REMARKS/ARGUMENTS

Claims

35 U.S.C. § 102(b) Rejection of Claims 1 and 9

The Office Action rejected Claims 1 and 9 under 35 U.S.C. 102(b) as being anticipated by Wang *et al.* (PNAS, 2003). The Office Actions stated: "Wang *et al.* teach the generation of mutant CaMKIIα proteins that are considered inactive in the presence of inhibitors specific for the mutant CaMKIIα mutants".

Applicants disagree with the conclusion of the Office Action that Wang *et al.* discloses "each and every" feature of Claim 1 and Claim 9, and at least of which is "a CaMKIIα gene of one or both of homologous chromosomes is substituted into an inactive type so that an inactive CaMKIIα, which has at least one amino acid residue modified in the catalytic domain of CaMKIIα"; and Traverse.

Claims 1 and 9 have been amended to include the features of Claims 3 and 4. Claims 3 and 4 have been Canceled.

The Office Action has incorrectly assumed the αCaMKII-F89G transgenic (Tg) mice described in Wang *et al.* (PNAS, 2003; page 4288, column 1, third paragraph, and Fig. 2) as being equivalent to the "knockin nonhuman animal" in Claim 1. However, Wang *et al.* does not disclose the substitution of a CaMKIIα gene into an inactive type so that an inactive CaMKIIα is expressed.

In the "knockin nonhuman animal" of Claim 1, a Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) gene of one or both of homologous chromosomes is substituted into an inactive type so that inactive CaMKIIα is expressed, and thereby the protein kinase activity of CaMKIIα is specifically impaired.

[CaMKIIα gene of homologous chromosome is substituted into an inactive type] =

↓ kinase activity

In contrast, the αCaMKII-F89G Tg mice of Wang *et al.* are not knockin mice but are transgenic mice overexpressing αCaMKII-F89G in the forebrain regions as a result of "αCaMKII-F89G transgene expression vector having αCaMKII promoter", which is constructed so that αCaMKII-F89G is expressed, being introduced into wild-type mice, as described in Fig. 2 (a) and page 4289, left column, third paragraph. Further, as shown in Fig. 2 (d) and (e), the αCaMKII-F89G Tg mice have higher CaMKII activity (Ca^{2+}-dependent and Ca^{2+}-independent CaMKII activities) than wild-type mice.

Wild type + [αCaMKII-F89G transgene expression vector having αCaMKII promoter] =

↑ kinase activity

This clearly indicates the Tg mice have not been modified so that an inactive CaMKIIα is expressed. In this respect, they are distinctly different from the "knockin nonhuman animal" of the present invention in which the protein kinase activity of CaMKIIα is impaired.

The Office Action appears to assume the αCaMKII-F89G Tg mice of Wang *et al.* are equivalent to the "knockin nonhuman animal" in Claim 1 where the protein kinase activity of CaMKIIα is impaired, since the CaMKII activity of the Tg mice is reduced to the wild-type mouse level by orally administrating an inhibitor (NM-PP1) to the Tg mice, that is, the CaMKII activity of the expressed mutant CaMKIIα is inactivated by the inhibitor (Fig. 2 (f)).

↑ kinase activity + [NM-PP1 inhibitor] = wild type

However, the protein kinase activity of CaMKIIα in the "knockin nonhuman animal" in Claim 1 (i.e., CaMKIIα is genetically inactivated) is impaired regardless of the presence or absence of the inhibitor (NM-PP1).

↓ kinase activity + [inhibitor] = ↓ kinase activity [no effect]

In this respect, the "knockin nonhuman animal" disclosed in the present application and claimed in Claim 1 is distinctly different from the αCaMKII-F89G Tg mice of Wang *et al.*,

which have protein kinase activity and CaMKII activity is enhanced above wild-type mice in the absence of the inhibitor (NM-PP1).

Even if the procedure of producing the αCaMKII-F89G Tg mice of Wang *et al.* is applied to the production of knockin mice (page 4292, left column, second paragraph), the resulting knockin mice would be expected to exhibit CaMKII activity equivalent to wild-type mice in the absence of the inhibitor, and therefore these mice are distinctly different from the "inactive knockin nonhuman animal" that is permanently inactivated as claimed in the present application.

Claim 9 claims a CaMKIIα knockin cell and has similar features as Claim 1. For the same reasons as discussed above for Claim 1, Wang *et al.* does not disclose "each and every" feature of Claim 9.

Therefore the presently claimed "CaMKIIα knockin nonhuman animal" of amended Claim 1 and presently claimed "CaMKIIα knockin cell" of amended Claim 9 are not anticipated by Wang *et al.*

The Examiner is requested to withdraw Wang *et al.* as a 102(b) Prior Art reference. In light of the foregoing arguments and amendments to the claims, the Examiner is respectfully requested to allow Claims 1 and 9.

35 U.S.C. § 102(b) Rejection of Claim 9

The Office Action rejected Claim 9 under 35 U.S.C. 102(b) as being anticipated by Hanson *et al.* (Neuron 1994). The Office Actions stated: "Hanson *et al.* teach the creation of a K42M or K42R catalytic domain mutants in CaMKIIα (termed α-CaMKII^{I}) that inactivate the kinase activity of the protein but retain the ability to multimerize and bind calmodulin."

Applicants disagree with the conclusion of the Office Action that Hanson *et al.* discloses "each and every" feature of Claim 9; and Traverse.

Hanson *et al.* teaches that cDNA encoding mutant CaMKIIα (α-CaMKII^{I}) in which the 42nd amino acid (Lys) is substituted by Arg (R) or Met (M) is introduced into COS cells to transiently express CaMKII^{I} (page 945, right column, lines 6-8, and the "Experimental Procedures" on page 953). A COS cell, which is a cell line cell derived from kidney cells, does not originally express CaMKIIα. CaMKIIα is specifically expressed in neuronal cells. In other words, Hanson *et al.* teaches transient expression of α-CaMKII^{I} in COS cells, which originally

do not express CaMKIIα, by exogenous introduction of α-CaMKII^{I}. However, Hanson *et al.* does not teach the knockin cells of the present invention, in which CaMKIIα is originally expressed and has been permanently substituted with the inactive type by genetic homologous recombination.

Claim 9 includes the feature "CaMKIIα gene of one or both of homologous chromosomes is substituted into an inactive type" [emphasis added]. Since a COS cell does not express CaMKIIα, the CaMKIIα gene cannot be "substituted" as claimed in Claim 9.

Therefore, Hanson *et al.* does not disclose "each and every" feature of Claim 9. The Examiner is requested to withdraw Hanson *et al.* as a 102(b) Prior Art reference. In light of the foregoing arguments and amendments to the claims, the Examiner is respectfully requested to allow Claim 9.

35 U.S.C. § 103(a) Rejection of Claims 1 – 4 and 7 - 13

The Office Action rejected Claims 1 – 4 and 7 – 13 under 35 U.S.C. 103(a) as being unpatentable over Elgersma *et al.* (Neuron, 2002), Wang *et al.* (PNAS, 2003), Hanson *et al.* (Neuron, 1994), and Sutoo *et al.* (Brain Res., 2002).

Applicants disagree with the conclusion of the Office Action and Traverse.

Claims 1 and 9 are independent claims with Claims 2, 7 and 8 dependent from Claim 1. As discussed above, Wang *et al.* does not disclose each and every feature of Claim 1 or Claim 9. The combination of Elgersma *et al.*, Hanson *et al.* and Sutoo *et al.* do not disclose the missing features of Wang *et al.* and therefore combination of the four references fails to make obvious Claims 1 – 4 and 7 – 13.

The Office Action concludes that the "knockin nonhuman animal" of Claim 1 is substantially described in Wang *et al.*, and based on this conclusion the Office Action believes that a person skilled in the art would have easily arrived at the present invention by using "α-CaMKII^{I}" (CaMKIIα in which the 42th Lys is substituted by Met or Arg) of Hanson *et al.* in either of the knockin mice of Wang *et al.* or Elgersma *et al.*

However, as described above, the mice of Wang *et al.* are not knockin mice, but are transgenic mice overexpressing αCaMKII-F89G in the forebrain regions by the introduction of the "αCaMKII-F89G transgene expression vector" into wild-type mice. Accordingly, even if "α-

CaMKII^{b}" of Hanson *et al.* is applied to the transgenic mice of Wang *et al.*, the "knockin nonhuman animal" and the "inactive CaMKIIα knockin cell" as presently claimed would not be made. The mice in Elgersma *et al.* are distinctly different from the "knockin mice" of Claim 1 in which the protein kinase activity of CaMKIIα is impaired.

Additionally, the mutant CaMKIIα of the CaMKIIα-TT305/306VA knockin mice in Elgersma *et al.* maintains protein kinase activity equivalent to that of wild-type mice (see page 494, right column, second paragraph of section "CaMKII Expression and Phosphorylation"). Therefore, protein kinase activity is not impaired as is claimed in Claims 1 and 9.

Furthermore, in the CaMKIIα-T305D knockin mice of Elgersma *et al.*, the calmodulin binding capacity is impaired and thereby the protein kinase activity of CaMKIIα is impaired (page 494, right column, second paragraph of section "CaMKII Expression and Phosphorylation"). In terms of the impaired calmodulin binding capacity, they are different from the "knockin mice" in Claim 1 in which the calmodulin binding capacity is maintained and the protein kinase activity is impaired.

Even if the amino acid substitution described in Hanson *et al.* is incorporated into the technique to produce mice described in Elgersma *et al.*, the "inactive knockin mice" of Claim 1 cannot be produced easily due to considerable technical difficulties. The CaMKIIα genomic gene is a huge gene extending more than 50,000 base pairs and consists of 18 exons (Nishioka *et al.*, FEBS Lett, vol. 396, pp. 333-336, 1996; see Table 1 on page 334 and Fig. 2 on page 335).

In contrast, gene fragments used in a targeting vector generally contain only several thousands of base pairs. Therefore, one skilled in the art recognizes that the genetic modification techniques directed to different functional domains of CaMKIIα encoded in different exons correspond to different techniques against entirely different genes.

In the knockin mice of Elgersma *et al.*, the targeting positions are autophosphorylation sites within the regulatory domain, which is located far from the targeting position of the "knockin mice" of Claim 1 in which protein kinase activity of CaMKIIα is specifically inactivated. Thr-305/306 targeted in the CaMKIIα knockin mice (TT305/306VA, T305D) of Elgersma *et al.* is encoded in exon 12, whereas Lys-42 targeted in the example of the present application is encoded in exon 2 (see pages 7 and 9, and Fig.1 of the specification). Exons 2 and 12 are located more than 20,000 base pairs apart from each other. Homologous recombination

techniques targeting different exons that are separated so far apart are comparable to those targeting totally different genes, which are accompanied by considerable technical difficulties, when compared with a gene recombination technique targeting different sites of a small-size gene.

Furthermore, the probability of the occurrence of homologous recombination in different gene sites varies depending on the genomic location, and the range and length of the targeting vector. Therefore, the "inactive knockin mice" disclosed in the present application cannot be easily achieved by simply combining the techniques described in the cited references.

Additionally, as for Claim 2, the Office Action cites Sutoo *et al.*, which teaches that CaMKIIα is highly expressed in the "nucleus accumbens", whereas CaMKIIα is moderately expressed in the "globus pallidus". The Office Action has interpreted the "globus pallidus" as "a component of the corpus striatum", and according to this interpretation has decided that it is within the expected range that the neuronal activity is greatly affected only in the nucleus accumbens, but not in the "corpus striatum" in the "inactive knockin mice" of the present invention.

This interpretation has a major flaw. Sutoo *et al.* clearly states that CaMKIIα is highly expressed in the "neostriatum" as well, which is the main portion of the "corpus striatum" (see last paragraph, left column on page7 and Fig. 4 on page 8). However, the Office Action ignores this point. The conclusion that Sutoo *et al.* discloses the features in Claim 2 is clearly incorrect because it is based only on the description about the "globus pallidus", which is only occasionally included in the "corpus striatum" and ignores the description of the "neostriatum", which is the main portion of the "corpus striatum". Moreover, the "corpus striatum" denotes "neostriatum", which includes "caudate-putamen", and does not include "globus pallidus" (see Figure 9 in present application and Yamagata Declaration).

The Office Action indicates the possibility that the "globus pallidus" might be included within the meaning of the "corpus striatum". There is confusion among researchers concerning which structures are included by the term "corpus striatum" (see "Carpenter's Human Neuroanatomy, 9th edition", Andre Parent, 1996, Williams & Wilkins; Chapter 19 "Basal Ganglia", page 795, right column, second paragraph, lines 9-17).

As disclosed in Sutoo *et al.*, CaMKIIα is widely distributed in the forebrain, especially in the hippocampus, cerebral cortex, striatum, nucleus accumbens, and amygdala. Therefore, changes in neuronal activity would be expected to occur in all of these areas in the inactive knockin mice. However, as claimed in Claim 2, the "knockin mice" only show a decline in neuronal activity in the nucleus accumbens with no changes in the cerebral cortex and striatum (see page 19 and Fig. 9 of the specification). These features of the "knockin nonhuman animal" of the present application are not predictable from the findings of the cited documents including Sutoo *et al.*

Therefore the presently claimed "CaMKIIα knockin nonhuman animal" in Claim 1 and "CaMKIIα knockin cell" in Claim 9 are not obvious over Elgersma *et al.*, Wang *et al*, Hanson *et al.* and Sutoo *et al.* Claims 2, 7 and 8 dependent from Claim 1 and thus are also not obvious over the cited references. Claim 3, 4 and 10 – 13 are Canceled.

The Examiner is requested to remove the combination of Elgersma *et al.*, Wang *et al*, Hanson *et al.* and Sutoo *et al.* as 103(a) Prior Art references. In light of the foregoing arguments and amendments to the claims, the Examiner is respectfully requested to allow Claims 1, 2 and 7 - 9.

New Claims 18 – 23

Claims 18 – 20 are dependent from Independent Claim 1 and Claims 21 – 23 are dependent from Independent Claim 9. Since Claims 1 and 9, as amended, are allowable over the cited references, Claim 18 – 23 are also allowable as being dependent from allowable claims.

In light of the foregoing arguments, the Examiner is respectfully requested to allow Claims 18 - 23.

Rejoinder of Claims 5, 6 and 14 - 17

Claims 5, 6 and 14 - 17 are currently withdrawn. As Claim 1 is allowable, as amended, and Claims 5, 6 and 14 - 17 contain all the features of the claim from which they depend, the Examiner is respectfully requested to rejoin Claims 5, 6 and 14 - 17.

In light of the foregoing arguments, the Examiner is respectfully requested to allow Claims 5, 6 and 14 - 17.

CONCLUSION

Claims 1 – 23 are Pending. Claims 1, 7 and 9 are Currently amended. Claims 2 and 8 are Previously presented. Claims 3, 4 and 10 -13 are Canceled. Claims 5, 6 and 14 - 17 are Withdrawn. Claims 18 – 23 are New.

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, arguments in support of the patentability of the pending claim set are presented above. In light of the above remarks, reconsideration and withdrawal of the outstanding rejections are specifically requested and it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

No additional fees are believed due; however, the Commissioner is authorized to charge any fees due in connection with the filing of this response to our Deposit Account No. 50-1349. If a fee is required for an extension of time under 37 C.F.R. § 1.136 that is not accounted for in the enclosed transmittal, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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